ANNUAL RESEARCH REPORT
California Olive Board and California Olive Oil Commission
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Project Title: Evaluation of new fungicides for control of olive leaf spot

Keywords: Fungicides, timing of applications, in vitro toxicity

JUSTIFICATION/ BACKGROUND

Review. Olive leaf spot or peacock spot, caused by the fungus *Venturia oleaginea* (syn. *Fusicladium oleagineum*, *Spilocea oleaginea*) (Fig. 1), is a sporadic disease of olive trees in California. In years with favorable environmental conditions, an orchard may up to 15% of its leaves and 10 to 20% of the fruiting twigs if the disease is not managed. Excessive leaf loss can also result in more olive knot because leaf scars are sites for bacterial infection. Symptoms most commonly develop on the leaf blade but are also found on petioles, fruit, and fruit peduncles (stems). At first, lesions are inconspicuous, superficial, sooty blotches. Later they become dark green to black circular spots containing mycelium and conidia (Fig. 2), and spots are surrounded by yellow halos (Fig. 2). These lesions resemble the spot on the tail of a peacock, and hence the name peacock spot. With numerous lesions, the leaf becomes chlorotic and falls.

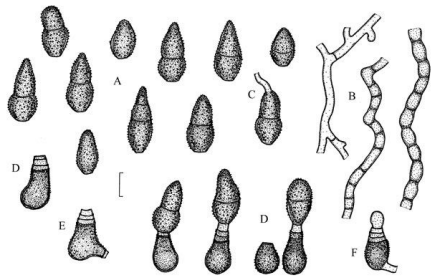

Fig. 1. *Venturia oleaginea.* A - conidia. B - superficial septate hyphae, C - germinating conidium, D - conidiogenous cells with several conspicuous annellations, E – percurrent proliferating conidiogenous cell, F - conidiogenous cell arising from a hypha. Scale = 10 µm.

Fig. 2. Range of symptoms of Peacock spot caused by *Venturia oleaginea.*

In California, lesions start forming in the fall and winter, but most disease develops in the spring. Leaves in the lower canopy where the humidity is higher are more severely affected, resulting in greater defoliation. Defoliated twigs often die later in the summer. Leaf infections occur on the upper surface and seldom penetrate beyond the epidermal layer. Once the leaf drops, however, the fungus colonizes the internal leaf tissues forming a dense mass of stromatic tissue. The sexual state of the pathogen has not been observed but molecular evidence indicates that the pathogen is a species in the genus *Venturia*. Olive cultivars vary in their susceptibility. Mission is the most susceptible followed by Manzanillo and to a lesser extent Sevillano, but all cultivars can become infected.

Leaf drop occurs mostly in late spring and summer. Infected leaves remaining on the tree start sporulating along the margins of lesions in the fall. Rainfall and wind-driven rain are the main dissemination methods; wind alone is not effective in detaching and disseminating conidia. Rainfall is essential for infections to occur regardless of the season. Temperature is important but often does not limit the development of the pathogen. High temperatures are more limiting to spore germination and mycelial growth than low temperatures. The optimum temperature for growth of the fungus is 21ºC, but growth can occur at 6ºC to 28ºC. The minimum duration of leaf wetness for spore germination is 48 h at 16ºC, 24 h at

20ºC, or 36 h at 24ºC. The incubation period for symptom development is 12 to 19 days over a temperature range of 10ºC to 25ºC.

Current Update: Currently available chemicals for managing the disease are copper and lime sulfur. Bordeaux mixtures or fixed coppers are commonly used to prevent copper injury. Lime sulfur can also eradicate the fungus in leaf tissues, but lime sulfur is difficult to work with and requires extra protective equipment for workers. Zineb is effective but is no longer available in the US. Timing of fungicide treatments in California include a postharvest application in late fall and an early spring application. Some research, however, has indicated that spring treatments are less effective. Use of copper treatments at these time periods corresponds with olive knot management timings. With more regulations concerning the use of copper (new copper limits for agricultural uses) and lime sulfur, alternative fungicides are needed that are highly efficacious and persist for extended time periods to prevent infections over the winter and spring when rainfall results in infection periods.

Based on results from our field studies, the multisite mode of action (MOA) fungicide Ziram and a pre-mixture of two single-site MOA fungicides, Inspire Super, were approved for residue trials at the IR-4 National Food Use Workshop in September 2018 for registration on olives. Strong support was provided based on the after-harvest and winter season usage with zero residues on the crop in the following harvest season as determined in subsequent IR-4 field and laboratory residue studies in 2019/2020. Ziram is a FRAC Code M3, whereas Inspire Super is a FRAC Code 3/9 fungicide. Thus, based on their multi-site MOA, the use of both products also provides an effective anti-resistance strategy. Continued research on these and other fungicides needs to be done to identify additional effective treatments and optimal use strategies. Thus, in 2020, we continued our field studies on the management of peacock spot. Fungicides included the chlorinated hydrocarbon chlorothalonil (FC M5), the polyoxin polyoxin-D (FC 19), the guanidine dodine (FC U12), and the organo-tin triphenyltin (FC 30), as well as a tank mixture of dodine and polyoxin-D, a DMI/anilinopyrimidine premixture (difenoconazole/cyprodinil - FC 3/9), and ziram (FC M3) in comparison to copper (M1) as the industry standard treatment. These products were tested in two field trials in Glenn and Sutter Co.

OBJECTIVES

1. Evaluate the performance of new and older fungicides in field trials.
    a) Dithiocarbamates (ziram), chlorinated hydrocarbons (chlorothalonil), and phthalimides (captan) (FRAC Codes – M3, M4, M5), DMIs (FC 3), polyoxins (FC 19), or mixtures such as FC 3/9, and FC 3 + 19.
    b) Evaluate proprietary fatty acids and surfactants to improve performance of fungicides.
2. Evaluate application timing of selected treatments.
    a) Fall, spring, or fall <u>and</u> spring.
3. Evaluate new fungicides for their in vitro activity.
    a) Determine the in vitro activity of selected fungicides that are effective in field trials.

PLANS AND PROCEDURES

1. *a,b. Evaluate the performance of new and older fungicides in field trials.* In studies in commercial Arbequina and Manzanillo olive orchards where the disease is known to occur, fungicides including ziram, chlorothalonil, dodine, polyoxin-D, and triphenyltin hydroxide or tank mixtures of dodine+polyoxin D or a premixture of difenoconazole/cyprodinil as compared to copper were applied using an air-blast sprayer. Untreated trees served as controls. Nu-Film P was used as the adjuvant to improve coverage and persistence of the fungicides. There were four replications for each treatment in a randomized complete block design. Disease incidence and severity were evaluated in late spring. Data were analyzed statistically using ANOVA and mean separation procedures of SAS 9.4.

2. *Evaluate application timing and adjuvants of selected treatments*. In field studies, selected fungicides were applied as fall and spring timings. Fatty acid products were not available for the study, and Nu-Film P was used as the adjuvant. There were four replications for each treatment in a randomized complete block

design for a factorial experiment. Disease incidence and severity were evaluated in late spring. Data were analyzed statistically using ANOVA and mean separation procedures of SAS 9.4.

3. *Evaluate new fungicides for their in vitro activity*. Isolates of the pathogen were obtained from several locations. To evaluate the in vitro toxicity of selected new fungicides with efficacy in field trials, the SGE method was used. Agar media were amended with fungicides in radial concentration gradients using a spiral plater. Suspensions of spores or mycelial strips were placed radially onto the amended media. This was done in an attempt to determine EC_{50} values for each fungicide/isolate using a computer program.

RESULTS AND DISCUSSION

1. a,b. Evaluate the performance of new and older fungicides in field trials. Treatments were applied in the fall (Nov. 2019) and winter (Feb. 2020) and were evaluated in April/June 2020 for peacock spot. The efficacy of fungicide treatments in managing olive leaf spot (peacock spot) on Manzanillo olive grown in a low-density orchard in Glenn Co. is shown in Table 1. All fungicides significantly reduced the disease as compared to the untreated control. Syllit, Inspire Super, Super Tin, and copper were highly effective reducing the disease to between 10.8% to 13.5% as compared to the control with 48.4% disease incidence. Ph-D, Ziram, Bravo, and the Syllit/Ph-D mixture were also very effective reducing the disease to between 16.3 to 16.5%. These data are similar to our 2019 trials where most of the same fungicides were evaluated. Disease levels were considered very high and the fungicides worked well under these conditions.

A second trial was done in Yuba Co. on Arbequina olive to evaluate the same fungicides in a high- density planting. The efficacy of fungicide treatments in managing olive leaf spot is shown in Table 2. Disease pressure was lower in this orchard with an incidence of 27.5% in the untreated control. All of the fungicides significantly reduced leaf spot incidence to <15.3%. Bravo was highly effective in this trial reducing disease incidence to 5%. Super Tin, Champ, Ph-D, Syllit, and Inspire Super also performed well, reducing the incidence to between 8 to 13%.

Table 1. Efficacy of fungicide treatments for management of peacock spot of Manzanillo olive, Glenn Co. 2019-2020.

				Applications		Incidence of disease**	
No.	Treatments*	Product rate/A	NuFilm-P	11-15-19	2-18-20	%	LSD^
1	Control	---	---	---	---	48.4	a
2	Ph-D	6.2 oz	12 fl oz	@	@	16.5	b
3	Ziram	128 oz	12 fl oz	@	@	16.3	b
4	Bravo	64 fl oz	12 fl oz	@	@	16.3	b
5	Syllit + Ph-D	32 oz + 6.2 oz	12 fl oz	@	@	16.3	b
6	Inspire Super	20 fl oz	12 fl oz	@	@	13.5	b
7	Super Tin	12 fl oz	12 fl oz	@	@	13.3	b
8	Syllit	48 oz	12 fl oz	@	@	13.0	b
9	Champ	128 oz	12 fl oz	@	@	10.8	b

*Treatments were applied using an air-blast sprayer at 100 gal/A.
** Disease was evaluated on 6-10-20 and 100 random leaves of each tree were assessed for the presence of typical disease symptoms.

Table 2. Efficacy of fungicide treatments for management of peacock spot of Arbequina olive, Yuba Co. 2019-2020.

				Applications		No. leaves diseased	
No.	Treatments*	Product rate/A	Nufilm-P	11-15-19	2-11-20		LSD^
1	Control	---	---	---	---	27.5	a
2	Ziram	128 oz	12 fl oz	@	@	15.3	b
3	Syllit + Ph-D	32 + 6.2 oz	12 fl oz	@	@	15.0	b
4	Super Tin	12 fl oz	12 fl oz	@	@	13.0	bc
5	Champ	128 oz	12 fl oz	@	@	12.0	bc
6	Ph-D	6.2 oz	12 fl oz	@	@	10.8	bc
7	Syllit	48 oz	12 fl oz	@	@	8.0	bc
8	Inspire Super	20 fl oz	12 fl oz	@	@	8.0	bc
9	Bravo	64 fl oz	12 fl oz	@	@	5.0	c

* Treatments were applied using an air-blast sprayer at 100 gal/A.
** Disease was evaluated on 6-10-20 and 100 random leaves of each tree were assessed for the presence of typical disease symptoms.

Ziram and the tank mixture Syllit/Ph-D were less effective than the other treatments with a leaf spot incidence of between 15.0% and 15.3%.

The registrant UPL has agreed to add olive to the polyoxin-D fungicide label. Because Ph-D is a biopesticide, it is exempt from tolerance and thus, no residue studies are needed. Label approval is expected in 2021. UPL is also the registrant of dodine, and this fungicide has European tolerances. IR-4 petitioned EPA through the Chemistry Science Advisory Council (ChemSAC) process to register dodine on olive without conducting residue studies because rates and tolerances are established in the EU. We prepared the request and justified the need for the IR-4 program submission. ChemSAC approved this request in Dec. 2020. Due to the small acreage of olive production limited mostly to California, registration of any new material will be limited to the IR-4 program. Therefore, Quadris Top was submitted to IR-4 in 2020. Because difenoconazole is an ingredient in both Inspire Super and Quadris Top, and residue trials were already done for Inspire Super on olive in the previous year, residue studies only need to be done for azoxystrobin.

With UPL (ziram, polyoxin-D, dodine) and Syngenta (difenoconazole/cyprodinil, or /azoxystrobin) supporting their respective products on olive, five new fungicide registrations will be an expected final outcome that will allow for sustainable olive leaf spot management programs for years to come.

2. Evaluate application timing of selected treatments. In our studies in 2019-2020, fall and winter applications were done for all treatments. This is because none of the treatments reduced the disease to very low levels.

3. Evaluate new fungicides for their in vitro activity. We are attempting to determine the in vitro activity of selected fungicides that are effective in field trials. This is very challenging because of the difficulty of growing the pathogen on laboratory media and because it has an extremely slow growth rate. These factors prevented us from using the spiral gradient and direct dilution agar assays. Additionally, the fungus only sporulates on leaves in winter and early spring under specific environments. Spore germination assays in the presence of fungicides will need to be developed and evaluated.

4. IR-4 GLP Studies. We have reviewed protocols, and we are assisting in field studies that are ongoing. Two fungicides, Ziram and Inspire Super, are currently in the system, and GLP field residue studies were conducted for both fungicides in 2020. The fungicide premixture of azoxystrobin and difenoconazole (Quadris Top) was nominated and approved as an A priority at the 2020 IR-4 Food Use Workshop that I attended in a virtual meeting. We will assist in developing protocols for field studies in 2021 for this latter fungicide. The registrant for triphenyltin (Super Tin) contacted us, and we conducted field trials in 2019/2020 on olive. The EPA, however, has placed a "red light" restriction with no additional registrations for the fungicide in the United States. This was discussed at the Food Use Workshop in September 2020. Thus, registration of Super Tin will not be pursued for olive or any other crop based on EPA concerns. Similar to Super Tin, registration of Topsin-M and Bravo will not be pursued for olive or any other crop based on EPA concerns (Table 3).

Table 3. Summary of new fungicides accepted into the IR-4 Program at the Food Use Workshop in September 2018-2020 and their registration status.

Year	Fungicide	Active Ingredient(s)	FRAC Code	IR-4/EPA	Status
2018	Ziram	ziram	M3	Supported	Ongoing
2018	Inspire Super	difenoconazole-cyprodinil	3/9	Supported	Ongoing
2019	Ph-D	polyoxin-D	19	Biopesticide	UPL Label change
2020	Quadris Top	difenoconazole-azoxystrobin	3/11	Supported	Initiated
2020	Syllit	dodine	U12	Supported	Chem-SAC
2018	Topsin-M	thiopphanate-methyl	1	Rejected	Not considered
2018	Bravo	chlorothalonil	M5	Rejected	Not considered
2020	Super Tin	triphenyltin hydroxide	M30	Rejected	Not considered